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Diversity of cyanobacteria and the presence of cyanotoxins in the epilimnion of Lake Yerevan (Armenia)

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ABSTRACT

This paper presents the first report of cyanobacteria and cyanotoxins from the South Caucasus region, in particular from Lake Yerevan (Armenia). *Microcystis, Dolichospermum* and *Planktothrix* were the key genera identified during the growing season. A trend of a remarkable increase in cyanobacterial densities was observed from 2012 to 2013 exhibiting bloom formation in June (by *Nostoc linckia*) with the highest values in June and August 2013, reaching up to $695.9*10^3$ cells mL^{-1}. Seasonal dependence of cyanobacterial density on temperature, and temperature as a driver for cyanobacterial cells growth and development were suggested. Biogenic nutrients were identified as co-drivers determining species richness and dominance, as well as the distribution of phytoplankton in different parts of the reservoir.

Cyanotoxin concentrations in the filtered biomass were reported during July 2012 for both stations of the reservoir (left and right bank). Microcystin-RR (MC-RR) was the most abundant and the most frequently observed cyanotoxin. Lower MC-LR concentrations were identified in all samples from both stations, with the highest values observed at the right bank in July 2012. [D-Asp^3]MC-RR, MC-YR, MC-HtyR, [D-Asp^3]MC-LR, MC-HilR, MC-WR, MC-LY and MC-LW were also identified in trace levels. Anatoxin-a (ANA) was reported in the samples from both stations during August 2012. Cylindrospermopsin (CYN) was present in trace concentrations in samples from both stations during July and in the sample from the left bank during September.

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1. Introduction

Climate change and eutrophication are the predominant phenomena stimulating bloom formations by cyanobacteria in freshwater ecosystems worldwide. Under different environmental factors the cyanobacterial community of a developed bloom may vary in species composition and dominance. Global warming is considered to contribute to the increased cyanobacterial growth and dispersion at the global scale (Paerl and Paul, 2012), since it could affect the thermal regime of lakes and increase their surface water temperature. As a consequence it could impair biological functions of aquatic organisms (Salmaso, 2010), change the structure of aquatic ecosystems and shift their trophic stage (Dokulil, 2013). Global warming results in higher water temperatures of the formed thermal column, which will likely become prolonged and more intense in the future (Gerten and Adrian, 2001). As higher water temperatures favor cyanobacterial dominance over phytoplankton communities of natural systems, they will be the first to benefit from the changing global climate (Salmaso, 2005).

Anthropogenic activities and related pollution sources, such as agriculture, industry, sewage, and road-runoffs are considered as

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the main factors to favor cyanobacterial proliferation. Although, changes in water temperature and external nutrient loading are the main factors influencing the trophic status of lakes, climatic conditions, morphometry, in-lake hydrodynamics and integrated hydrological changes, such as low turbulence, stagnant water conditions, and increased pH values, as well as foodweb structure, are other important factors that could affect the development of cyanobacterial blooms and change their species variety (Paerl and Huisman, 2008).

The increasing presence of cyanobacteria in surface water bodies is an emerging environmental issue, mainly due to the ability of some cyanobacterial strains to synthesize and release cyanotoxins (Chorus and Bartram, 1999; WHO, 2003). Their occurrence may induce health problems to animals and humans, since freshwaters could be used for human consumption, irrigation, fishing, as well as recreational activities (Chorus and Bartram, 1999). Therefore, certain guidelines have been implemented in order to control cyanobacterial cell density and cyanotoxin concentrations in waters used for certain purposes (Farrer et al., 2015; WHO, 2003). The main concept of the guidelines is to properly assess the potential and the actual risk originating from cyanobacteria, namely cyanobacterial cell count and concentration of cyanotoxins in water.

Cyanotoxins pose a significant risk to human health (Sivonen and Jones, 1999). They are classified based on their toxic effects as hepatotoxins (e.g., microcystins, nodularins, cylindrospermopsin), neurotoxins (e,g., anatoxin-a, saxitoxins, β-methyl-L-alanine BMAA) and dermatotoxins (lyngbyatoxin, aplysiatoxin) (Carmichael, 2001; Merel et al., 2013). Microcystins (MCs) are cyclic heptapeptides (approximately 248 identified variants) with a molecular weight of approximately 1000 g/mol (e.g. 995.17 for MC-LR and 1038.2 for MC-RR, respectively) (Spoof and Catherine, 2017). They all share the common chemical structure of cyclo(-D-Ala-L-X-D-MeAsp-L-Z-Adda-D-Glu-Mdha), where X and Z are varying L-amino acids. Adda is (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl- 10-phenyldeca-4,6-dienoic acid, D-MeAsp is D-*erythro*-β-methyl-aspartic acid, and Mdha is N-methyl-dehydroalanine (Carmichael et al., 1988). MCs are hepatotoxins also inducing cytotoxicity via phosphatase activity inhibition (Sivonen and Jones, 1999).

The alkaloid (+) Anatoxin-a (ANA), is a bicyclic amine (2-acetyl-9-azabicyclo[4,2,1]non-2-ene) known for its acute neurotoxicity (Van Apeldoorn et al., 2007), with a molar mass of 165 Da. The primary mechanism of its effect is potent and prolonged nerve depolarization with further prevention of impulse transmission and possible paralysis, asphyxiation, and death (Sivonen and Jones, 1999).

Cylindrospermopsin (CYN) is a cyclic guanidine alkaloid, with a molar mass of 415 Da (Chorus and Bartram, 1999; De La Cruz et al., 2013). CYN targets the liver, kidney, spleen, thymus and heart through the primary mechanism of inhibition of protein synthesis (Metcalf and Codd, 2012). Cyanobacteria associated with the production of various cyanotoxins have been thoroughly presented by Bernard et al. (2017).

Lake Yerevan is a part of Lake Sevan - Hrazdan River - Lake Yerevan water cascade that was built with the purpose to protect the Yerevan city against flooding and to balance the humidity level of south-western part of the city. Moreover, it was meant to be used as a public water supply, for irrigation, fishing activities, etc.

There is a growing concern about the possible effect of cyanotoxins to the Yerevan ecosystem and human health. The main scopes of this study were to evaluate the current trophic status of Lake Yerevan, to assess the potential risk of developed cyanobacteria blooms and to evaluate for the first time their cyanotoxin content. Another aim of this study was to assess the quantitative and qualitative distribution of cyanobacterial species in water originating from the studied area. Obtained results could serve as a tool for water administration and public health authorities to develop more effective methods for water management, to prevent the formation of algal blooms and to propose possible treatment procedures.

2. Materials and methods

2.1. Study site

Lake Yerevan, located at 40°9′35.04″N and 44°28′36.54″E (Fig. 1), is an artificial water body in the southwestern part of the capital of Armenia. It exhibits high residence time, typical low mixing and summer stratification with thermal column formation. Its surface area is 0.65 km^2, with a shoreline of 6.3 km, and maximal width in 5 km. The maximum depth is 22 m, and the surface elevation is 908 m. The maximum permitted level of the Lake water may reach 895 m (level manipulation ~13 m annually). Water temperature ranges from +1 °C up to +28 °C. During warmer seasons water transparency drops from 2 m up to 0.5 m. Water pH ranges from 7.8 to 8.4.

Lake Yerevan is a part of Lake Sevan - Hrazdan River - Lake Yerevan water cascade that continues as River Hrazdan joining River Arax in the Ararat valley, along with Armenian-Turkish borders. Afterwards it flows along the Armenian-Iranian and Iranian-Azerbaijan borders to meet with the Kura River and inflow into the Caspian Sea. The initial volume of the Lake Yerevan reservoir after its construction was about 0.005 km^3. During October 2006–June 2007 the reservoir volume reached 0.004 km^3 level (USAID, 2006), demonstrating high sedimentation. Lake Yerevan has lost up to 25% of its initial volume, due to inappropriate management of water masses since the Soviet times and sedimentation. Moreover, due to seasonal water level manipulations (up to 13 m annually), the reservoir of Lake Yerevan suffers of annual surface area revertible decline, approximately up to 5%. A similar issue was also observed with the Aral Sea that was steadily shrinking since the 1960s after the inflowing rivers were diverted by the Soviet Union irrigation projects.

2.2. Sampling stations

All samples (Fig. 1) were collected monthly from surface waters (1 L of water in sterile sampling bottles from euphotic zone - up to 50 cm depth) of Lake Yerevan (X 1 station - left bank and X 2 station - right bank, up to 5 m distance from the shoreline - called “inshore” hereinafter) during the three growth seasons (spring/summer/fall, May–October) in 2012 and 2013. In 2014 water samples were collected from additional stations (X 3 station - entering part of River Hrazdan - “entry” hereinafter, X 4 station - center and X 5 outflow of Lake Yerevan - “exit” hereinafter, as well as X 6 and X 7 up to 5 m distance (10 m from shoreline) - “nearshore” hereinafter, from stations X 1 and X 2). Water samples were preserved with ethanol (10% final concentration) in order to examine the seasonal cyanobacteria species richness, as well as their abundance.

2.3. Chemicals and reagents

[D-Asp^3]MC-LR, [D-Asp^3]MC-RR, MC-WR, MC-HtyR, MC-HilR, MC-LY, MC-LW and MC-LF standards were supplied by ENZO Life Science (Lausen, Switzerland). MC-RR, MC-LR, MC-YR, MC-LA and Nodularin standards were supplied by Sigma-Aldrich (Steinheim, Germany). CYN was purchased from Abraxis (Warminster, UK) and ANA-fumarate from TOCRIS Bioscience (Bristol, UK). All substances had purity >95%. Methanol (MeOH) of HPLC grade (99.9%) and

Fig. 1. The map of Lake Yerevan, illustrating the sampling points.

dichloromethane (DCM) of analytical reagent grade (99.9%) were obtained from Fischer Scientific (Leics, UK), acetonitrile (ACN) for HPLC (≥99.9%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). High purity water (18.2 MOhm cm at 25 °C) was produced on-site, using a Temak TSDW10 system (Carlsbad, USA). Formic acid (FA) (>98%) was purchased by Riedel-de Haën (Seelze, Germany).

2.4. Biological observations

Partial processing of the samples was carried out at the department of Aquatic Ecology, Ludwig-Maximilians University, Munich, Germany. Identification of cyanobacterial community species' richness was carried out using various handbooks and guidelines (Linne von Berg and Melkonian, 2000; Streble and Krauter, 2001; www.algaebase.org; www.algalweb.net). The quantitative analyses of the water samples have been performed in the Utermöhl sedimentation chambers under the inverted microscope (Utermöhl, 1931). Twenty randomly chosen fields with three replicates per sample were counted to minimize the counting error <10% (Lund et al. 1958). The abundance of cyanobacterial species was calculated by direct cell counting under the microscope. The numbers of cells fitting into the counting chamber were counted for the following genera: *Microcystis, Dolichospermum, Planktothrix, Merismopedia, Oscillatoria, Pseudanabaena. Arthrospira* (former *Spirulina*) was counted as one unit as well as the remaining cyanobacteria.

The software package Sigma-Plot (Version 12.5) was used for data analysis and for graphic illustration of the results.

2.5. Cyanotoxin analysis

To quantify the cyanotoxin content in Lake Yerevan, sampled water from the reservoir was filtered over Whatman GF/F filters. The filters were dried and stored at −18 °C. During June 2012, approximately 100 mL water (6.5–7.5 mg of cyanobacterial dry weight) were filtered for each sample. During July–September 2012 approximately 250 mL water (9.0–18.5 mg of cyanobacterial dry weight) were filtered.

For the extraction of cyanotoxins from filtered biomass, the following procedure was applied: filters were placed in Eppendorf tubes, followed by the addition of 9 mL extraction solvent containing 75% MeOH: 25% water and were subsequently sonicated in an ultrasound water bath for 15 min to achieve cell lysis. The extract was centrifuged at 4000 rpm for 10 min at 20 °C and the supernatant was collected and filtered through 0.22 μm PVDF filters (Agilent). 3 mL of the filtrate was collected, dried under a gentle nitrogen stream and reconstituted with 500 μL of a solution containing 95% water and 5% MeOH. The extracts were analyzed for CYN, ANA and MCs ([D-Asp^3]MC-RR, MC-RR, MC-YR, MC-HtyR, [D-Asp^3]MC-LR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF) using the LC-MS/MS method described by Zervou et al. (2017a). A Finnigan Surveyor LC system, equipped with a Finnigan Surveyor AS autosampler (Thermo, USA), was used to separate the target analytes. Detection and identification was achieved using a Finnigan TSQ Quantum Discovery Max triple-stage quadrupole mass spectrometer (Thermo Fischer Scientific, USA), with electrospray ionization (ESI). Multiple Reaction Monitoring (MRM) mode was selected for the detection of the compounds.

For samples of November 2016, the Whatman GF/F filters with Lake Yerevan water (1 L) were processed in 40 mL clean glass flasks by double extraction (1 h each extraction) with addition of 20 mL extraction solvent of 75% MeOH: 25% water, containing 0.2% acetic acid, filtering the extract into the new glass and further vacuum dry overnight and redissolved in 5 ml of 50 mM phosphate buffer. Analysis of those aliquots was carried out by ELISA (Abraxis PN 520011) using a BioTek ELx808 ELISA reader.

2.6. Isolation and purification of cyanobacterial culture

Cyanobacterial single colony and filament have been isolated directly from water samples of Lake Yerevan from November 2016 into BG-11 and WC media. Some cultures were isolated from agar plates. Cultures incubation was performed in a Percival incubator at 24 °C at a moderate light intensity (~50 μEinsteins; 8 h light/16 h dark life cycle).

3. Results

3.1. Physico-chemical parameters of Lake Yerevan waters

Values of N and P concentration in Lake Yerevan waters were obtained from the Ministry of Nature Protection, Monitoring Center, Republic of Armenia, (www.armmonitoring.am/). The results are public and they are obtained by the formal water

authority. During 2012–2013 the maximum concentration of nitrite ions reached the level of 0.33 mg N L^{-1} (June 2013). Mean annual values of nitrite ions for 2012 and 2013, were 0.092 and 0.12 mg N L^{-1} and they exceeded the maximum allowed concentration MAC (0.02 mg N L^{-1}) by 4.6 (2012) and 6.0 (2013) times, respectively. The MAC is the concentration of nutrients to consider water at mesotrophic stage, after which the water body is considered eutrophic. The annual mean concentrations for nitrate and phosphate ions during 2012–2013 were found to be lower than their MAC. During 2014 however, they increased reaching the "IV" class – eutrophic, according to the National Standards of water quality (Ministry of Nature Protection, 2011). The maximums of the TN/TP ratio were recorded during August of each year and they were found to be > 90, >170 and > 70, for 2012, 2013 and 2014, respectively. Ammonium ions reached a maximum of 1.8 mg N L^{-1} during 2012–2013. Mean annual concentrations of NH_4^+ during 2012 and 2013 were 1.25 and 0.78. They exceeded the MAC (0.39 mg N L^{-1}) by 3.2 (2012) and 2.0 (2013) times, respectively. Specifically during August 2013, NH_4^+ concentration was 4.7 times (August) higher than the MAC. The mean value of dissolved oxygen from May to October 2012 were higher than 5.0 mg O_2 L^{-1}, but in 2013–2014 the same parameter was recorded to be in range of 4.0–5.0 mg O_2 L^{-1}.

3.2. Density of cyanobacteria in Lake Yerevan waters

The analysis of cyanobacterial density in Lake Yerevan waters demonstrated increased values in all samples collected from the inshore part of the right bank (X 2). Relatively low values of abundance occurred in the spring and autumn months of all studied years. Data for total density during 2012 (Fig. 2a) were in similar range with values of 2014 (see Fig. 2c), with an increase during the warmer seasons. The highest values were observed during 2013 (see Fig. 2b) with early summer bloom formation tending to reduce compared to the same period of 2012 and 2014. The maximum total abundance (695.9 * 10^3 cells mL^{-1}) occurred in June 2013, which tended to decrease to 16.83 * 10^3 cells mL^{-1} (X 1) in fall season (Fig. 2 b, c). For the inshore stations (X 1 and X 2), values of abundance were in similar range during August 2012 (Fig. 2 a). In May 2013 the observed abundances for X 1 and X 2 stations differed more than two times. In June 2013, when the highest value was observed at the X 2, the value of X 1 was about 1.3 times less.

In 2014, the cyanobacterial community of Lake Yerevan was characterized by relatively low values of abundance in the epilimnion and nearshores (0.15–38.3*10^3 cells mL^{-1}) (see Fig. 2 c and Fig. 3). The higher values were again observed for the samples from in- and nearshore stations (X 1 and X 2, X 6 and X 7), with clear dominance at the right bank stations (Fig. 3). Mean values for 2013 are approximately 60 times higher than mean values for 2012 and 2014. In general, quantitative data of cyanobacterial community during 2014 demonstrate reverse direction of the Lake trophic stage back to mesotrophy.

3.3. Species richness in cyanobacterial community of Lake Yerevan waters

In total 27 cyanobacterial species were identified. Cyanobacteria contributed with 14 species for 2012 and 18 species for 2013 (see Fig. 4a and b). In 2014 cyanobacteria shared higher species richness, compared to 2012 and 2013 (24 species) (see Fig. 4c). The domination pattern of observed species changed slightly between different seasons and years for the analyzed stations. Cyanophyta, dominating in all samples from in- and near-shore samples, were represented mainly by *Microcystis* spp. (with *M. aeruginosa* and *M. ichthyoblabe*). Another taxa of Cyanophyta observed for the all samples, were *Dolichospermum* spp. (with *D. circinalis, D. flos-aquae* and *D. planktonica*) and *Planktothrix* sp. f. *gomontii* (with *P. agardhii*). *Aphanothece* and *Aphanocapsa* were observed in relatively high numbers. In June 2013, *Nostoc linckia* was observed in large numbers to form a water bloom in inshore waters with highest numbers at the right bank. *Spirulina abbreviata* was only observed during May 2013 (X 1). *Merismopedia elegans* was registered in low numbers in the samples obtained in May 2012 from X 1 station and in May, June and July 2014, for both samples from the left bank (X 1 and X 6) and the center. It was not observed during the growth period of 2013.

3.4. Occurence of cyanotoxins in Lake Yerevan filtered biomass

The presence of cyanotoxins in biomass filtered from water samples during June–September 2012 for X 1 and X 2 stations was also investigated using LC-MS/MS (Table 1). According to the obtained data, 12 cyanotoxins were detected with the highest cyanotoxin content observed in biomass from both stations in July. Various MCs have been identified. MC-RR was the predominant toxin identified (concentrations reaching up to 34.8 ng mg^{-1} dw). MC-LR was also identified during the same period in samples from both stations at lower concentrations. The highest concentration of MC-LR was found for X 2 in July (5.0 ng mg^{-1} dw). During the same period for X 1 and X 2, MC-WR was also identified at concentrations 3.8 and 2.4 ng mg^{-1} dw, respectively and [D-Asp^3]MC-RR at concentrations 1.6 and 1.1 ng mg^{-1} dw, respectively. Most targeted cyanotoxins except for Nodularin, MC-LF and MC-LA have been identified in some samples. In August, ANA was found in both samples (2.3 and 1.2 ng mg^{-1} dw, for X 1 and X 2, respectively). CYN was found in trace concentrations in the samples (concentration below LOQ) in both stations for July and in the sample from X 1 for September.

Recent investigations using ELISA for the determination of MCs, showed detectable concentration of total MCs in November 2016 in water samples of the right bank (<LOQ), meanwhile the MCs concentration for left bank, as well as in samples for 5 m and 10 m depth of central part of Lake Yerevan were below the detection limit (0.8 ng L^{-1} total MC concentration).

Analysis of MCs concentration using ELISA in pure cultures demonstrated the following results: for genus *Planktothrix* – 0.109 ng mg^{-1} dw, for genus *Fischerella* – 0.159 ng mg^{-1} dw, for genus *Oscillatoria* 0.002 ng mg^{-1} dw and for genus *Pseudanabaena* – 0.238 ng mg^{-1} dw. Concentration of MCs in the culture of genus *Cyanobium* was below the detection limit.

4. Discussion

4.1. Temperature changes and cyanobacterial production

The seasonal manipulations in the water level of Lake Yerevan (up to ±13 m) make the Lake basin a unique study area. Due to low water mixing and wider thermal gradient the central part of Lake Yerevan is stratified from April to October. In summer, water transparency may drop from 2 m as low as 0.5 m, which is accompanied by decreased water mixing and thermal gradient, resulting in thermal column stability. Additionally, these changes stimulate an elevated temperature gradient in the reservoir (from +7 °C in October up to +28 °C in August).

During 2012–2014 the cyanobacterial community of Lake Yerevan was split into three main zones based on the temperature differences up to 0.5 °C. The area with the highest density of cyanobacteria and lower species richness, is the right bank (warmer, drier and sunnier), with *Microcystis* and *Dolichospermum*

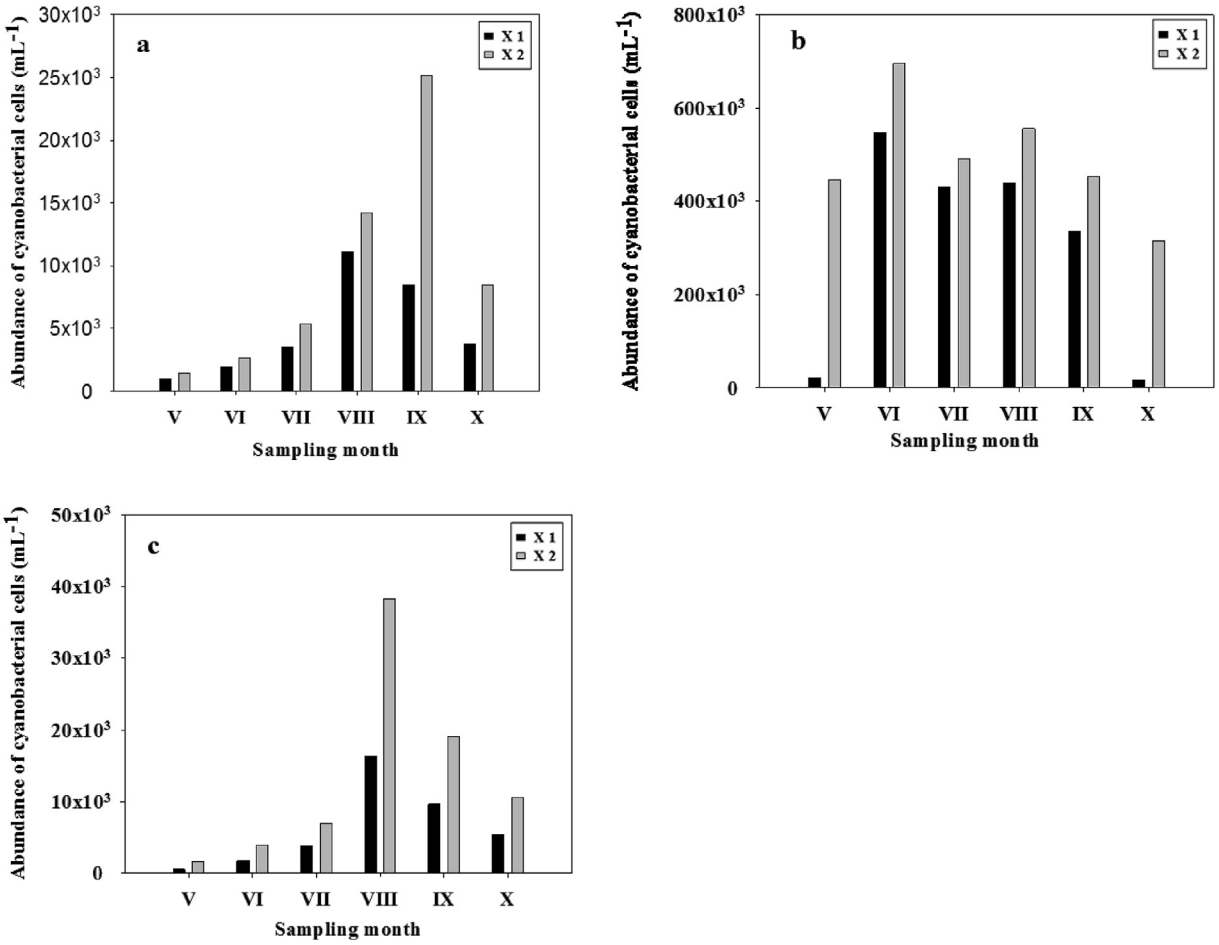

Fig. 2. Total abundance of cyanobacteria in inshore waters (left bank - X 1, right bank - X 2) of Lake Yerevan (cells mL^{-1}) during (a) 2012, May–October, (b) 2013, May–October and (c) 2014, May–October.

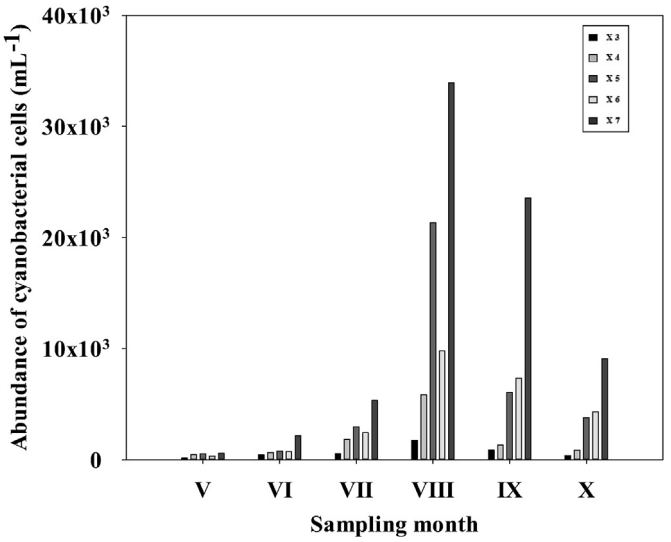

Fig. 3. Total abundance of cyanobacteria in euphotic zone of Lake Yerevan in May–October of 2014 (cells mL^{-1}) (entry - X 3, center - X 4, near-shores - X 6 - left bank, X 7 - right bank, and exit - X 5).

dominating the community. The second area that is less warm, more wet and not sunny (left bank), was subdominated by *Aphanothece* and *Aphanocapsa,* together with *Microcystis* and *Dolichospermum* genera. Finally, the third part of the Lake (closer to the center and nearshore parts), was subdominated by *Planktothrix* group together with *Microcystis* and *Dolichospermum* genus. During cold seasons, the cyanobacterial abundance in the epilimnion of the central part nearshore stations remains low, but during warmer seasons, higher richness of density and selective dominance of species is observed. This suggests that cyanobacteria are season-dependent and temperature is a regulating co-factor for cyanobacterial growth and development in Lake Yerevan.

According to Edwards et al. (2013), Jöhnk et al. (2008) and Lürling et al. (2013), a thermal gradient may explain most of the variation in the occurrence of *Microcystis* spp. Dokulil and Teubner (2000) have included *Microcystis* spp. in the group of cyanobacteria, preferring high water column stability, as in our case. Kromkamp et al. (1988) demonstrated decreasing buoyancy for *M. aeruginosa* in cold waters. Nixdorf et al., 2003 included *P. agardhii* in a group of turbulent species with a higher competitive capacity in turbid temperate lakes. Post et al. (1985) found *Planktothrix* sp. to grow well at temperatures of 10 °C or lower with decreased buoyancy in cold waters (Foy, 1983). Carey et al. (2012) recognized a genus such as *Planktothrix* to benefit from reduced light in the water column and therefore, it can proliferate over competitors, e.g. *Microcystis*. These observations related to temperature gradient might be the most reasonable explanation of registered increase in *P. agardhii* density in the epilimnion of Lake Yerevan for cold seasons.

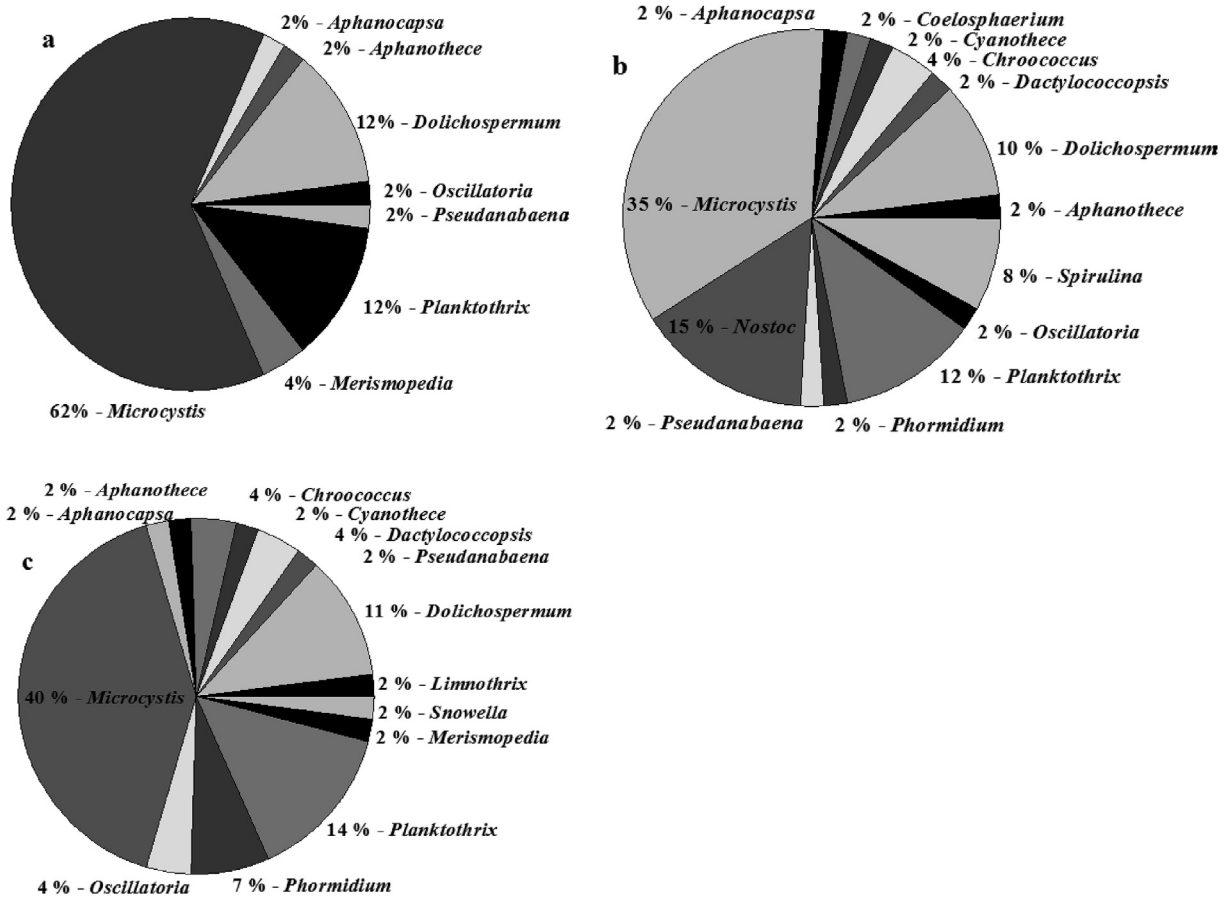

Fig. 4. Density-based proportional contribution of the main cyanobacteria genera of the cyanobacterial community in epilimnion of Lake Yerevan during (a) 2012, May–October, X 1 and X 2 stations, (b) 2013, May–October, X 1 and X 2 stations and (c) 2014, May–October, X 1 - X 7 stations.

Table 1
Cyanobacterial toxins in Lake Yerevan filtered biomass (ng mg^{-1} dry weight dw) during June–September 2012 using LC-MS/MS.

Sample station	Month	Cyanotoxin concentration (ng mg^{-1} dry weight dw)											
		CYN	ANA	[D- Asp^3]MC-RR	MC-RR	MC-YR	MC-HtyR	[D- Asp^3]MC-LR	MC-LR	MC-HilR	MC-WR	MC-LY	MC-LW
X 1	June	ND[a]	ND	<LOQ[b]	1.8	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND
X 2	June	ND	ND	<LOQ	1.7	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND
X 1	July	<LOQ	ND	1.6	34.8	<LOQ	ND	ND	3.0	<LOQ	3.8	ND	ND
X 2	July	<LOQ	<LOQ	1.1	22.6	<LOQ	<LOQ	ND	5.0	<LOQ	2.4	<LOQ	ND
X 1	August	ND	2.3	ND	0.3	ND	ND	ND	<LOQ	ND	ND	ND	ND
X 2	August	ND	1.2	ND	0.4	ND	ND	ND	<LOQ	ND	ND	ND	ND
X 1	September	<LOQ	ND	ND	1.6	ND	ND	<LOQ	2.3	<LOQ	ND	ND	<LOQ
X 2	September	ND	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND
LOD		0.1	0.1	0.2	0.1	0.4	0.5	0.3	0.3	0.5	0.4	0.5	0.4
LOQ		0.3	0.3	0.6	0.3	1.2	1.5	0.9	1.0	1.5	1.2	1.5	1.2

[a] **ND:** Not Detected < LOD (Limit of Detection).
[b] **< LOQ:** Values lower than LOQ (Limit of Quantitation) (i.e. detection confirmed but cannot be accurately quantified at that level).

4.2. Relation of trophic status to cyanobacterial proliferation

Chemical data of high nitrogen and phosphorus, together with decreased oxygen content for summer months, demonstrate that Lake Yerevan corresponds to the stage of meso-to eutrophy. Also increased TN/TP in Lake Yerevan (reaching the maximum value TN/TP > 170 by weight), most probably promotes cyanobacteria proliferation in summer with dominance of *Microcystis* sp. (*M. aeruginosa* and *M. ichthyoblabe*), *Dolichospermum* sp. (*D. circinalis, D. flos-aquae* and *D. planktonica*) and *P. agardhii*. The abnormally high TN/TP ratio, which is more typical for eutrophic waters, was observed only during August 2013, when Armenia experienced very low annual precipitation. High air temperature and consequently increased water temperature resulted in water intensified evaporation (Ministry of Emergency Situations, Republic of Armenia).

In June 2013, *N. linckia* has formed water blooms along with high numbers of *Microcystis* and *Dolichospermum*. The real cause for the observed rapid growth of *N. linckia* (not observed afterwards) was not revealed. Presumably, there were some changes in the trophic status of Lake Yerevan that coincided with the first prompt rise of cyanobacterial cells, starting in September 2012. A similar prompt

rise in *D. flos-aquae* density, with surface scum formation, was observed in September 2012 in Lake Sevan by (Minasyan, unpublished results). Further decrease of total cyanobacterial density in July and reverse dominance by *M. aeruginosa* in August was attributed to high nitrogen content and phosphorus limitation.

In the past, Smith (1983) and Steinberg and Hartmann (1988) have demonstrated certain cyanobacteria, such as *Microcystis*, *Anabaena*, *Oscillatoria*, to proliferate under favorable TN/TP ratios of 20/1 (by weight). *Microcystis* benefits in P-limiting conditions over other cyanobacterial genera (Hecky and Kilham, 1988) due to its capacity to accumulate P in polyphosphate bodies - volutin granules (Jacobson and Halmann, 1982). Reynolds demonstrated growth dependence of *Dolichospermum* over nutrients concentrations being higher than over temperature (Reynolds et al., 2002). According to (Post et al. (1985); Wagner and Adrian, 2009), *Dolichospermum,* together with *P. agardhii* prefer habitats of highly eutrophic conditions and they are more sensitive to nutrients concentration (especially to P), compared to *Microcystis*. These studies are in accordance to our findings, since in our case, *Microcystis* and *Dolichospermum* proliferated under high TN/TP ratios. Moreover, Ganf and Oliver (1982), Ibelings and Maberly (1998) as well as Brookes and Ganf (2001), suggested that *M. aeruginosa* and *Dolichospermum* sp. - are highly buoyant and adapted to alternating periods of mixing, and are able to migrate to the hypolimnion and access the nutrients trapped in the lake bottom, which gives them an advantage over the non- or slowly-migrating species (Carey et al., 2012). Genus *Merismopedia* (*M. elegans*), which is classified as tolerant to changes in nutrient richness (Reynolds et al., 2002), appeared in summer 2012 in X 1 station and in X 1, X 4 and X 6 stations in 2014, but it was not found in 2013. *Pseudanabaena limnetica (P. limnetica)* was equally numerous at medium level of cyanobacterial community for mainly summer samples. *Limnothrix redekei* and *Snowella lacustris* occurred with low frequency in the samples for X 4 - X 7 stations in May–September 2014. *S. abbreviata* was observed in May 2013 in left bank station. Additionally to this, *P. formosum* tended to increase in density during 2014. It is obvious that the occurrence and long-lasting dominance of certain cyanobacterial species in Lake Yerevan is specific for warm periods, presumably due to Lake Yerevan being influenced by various environmental factors, which may stimulate certain cyanobacterial community dominance.

Past investigations in 2003–2006 of inshore waters (X 2 - right bank) of Lake Yerevan (Stepanyan et al., 2006, 2011) demonstrated dominance of phytoplankton community by species *D. flos-aquae* and *Aphanizomenon flos-aquae (A. flos-aquae)* with maximum abundance in August up to $3.12*10^3$ cells mL^{-1}, which is quite lower than the results obtained during our investigations. One of the reasons might be the fact that in 2006–2007, after performing a USAID-funded investigation (USAID, 2006), the reservoir was completely dried off to remove the bottom sediments and afterwards filled with waters of River Hrazdan. Besides that, water and air average temperatures have increased in the last years (Ministry of Emergency Situations, Republic of Armenia) probably due to climate change. Although in the past, Lake Yerevan had been characterized as mesotrophic (Stepanyan et al., 2006, 2011), seasonal successions (2012–2014) of algal species composition (Minasyan, 2016) and their density together with bloom formation in 2013, indicate that Lake Yerevan trophic status gradually led towards eutrophication. The tendency of Lake Yerevan trophic evolution in 2014 had demonstrated reverse direction to mesotrophic stage.

4.3. Cyanotoxin occurrence

Our results of the first report of cyanobacteria and cyanotoxin presence in surface waters originating from the area of South Caucasus, indicate the presence of cyanotoxins belonging to different chemical groups (CYN, ANA and MCs) including different analogues of the same group (e.g. [D-Asp^3]MC-RR, MC-RR, MC-LR and MC-WR). MC-RR and MC-LR were the predominant toxins found, at concentrations reaching 34.8 and 5.0 ng mg^{-1} dw, respectively. The highest production of toxins occurred during warm periods (July, August). A wide variety of toxins was detected, although their concentration levels were not high.

The concentration of MCs found in our study are significantly lower compared to several investigations related to the presence of cyanotoxins in surface waters from South-East Europe (Table 2). In the case of water bodies located in Bulgaria, maximum values for total MCs were found to be 1070 ng mg^{-1} (Pavlova et al., 2006; Stoyneva-Gärtner et al., 2017). Cyanotoxin concentrations in Greek water bodies have been found to reach 754 ng mg^{-1} and 457 ng mg^{-1}, for MC-RR and MC-LR, respectively (Zervou et al., 2017b). In Romania (Boaru et al., 2006) total MCs ranged from 199 to 2875 ng mg^{-1}, much higher compared to the present study. Furthermore, studies on the presence of cyanotoxins in water bodies located in Serbia, Russia and Turkey, also indicate higher concentrations compared to the present study of Lake Yerevan. Surface water bodies located in South-East Europe have a proven potential to produce Harmful Algal Blooms and increased cyanotoxin content. Therefore, despite the low cyanotoxin concentrations found in Lake Yerevan, there is a necessity for frequent and reliable monitoring of the Yerevan water cascade system.

Occurrence of cyanotoxin producing cyanobacteria in various water resources has been demonstrated worldwide (Pelaez et al., 2010). Under certain circumstances, cyanotoxins can reach high concentrations in surface water and might pose health and ecological risks (Codd et al., 2005a; b). Guideline values for cyanobacteria and MCs present in waters used for recreational purposes have been set by the World Health Organization (WHO, 2003; Farrer et al., 2015). A guideline value of 20000 cells mL^{-1} stimulating synthesis up to 10 μg L^{-1} of total MCs, corresponds to low probability of adverse health effects. 20000-100000 cells mL^{-1} correspond to moderate probability and >100000 cells mL^{-1} correspond to high probability of adverse health effects. Pilotto et al. (1997) suggested a guideline level of $20*10^3$ cyanobacterial cells mL^{-1} (with 2–4 μg MC L^{-1} being expected in case of MC-producing genera dominance up to 10 μg L^{-1}).

Based on the cyanobacterial cell content found in our samples (WHO, 2003), during 2013 Lake Yerevan can be assessed at potential high risk level ($695.9*\ 10^3$ cells mL^{-1}) (see Fig. 2). During October 2012, based on the cyanobacteria cell content, Lake Yerevan showed low to moderate risk of adverse health effects, according to WHO guidelines for waters intended for recreational activities. The calculated total concentrations of MCs, expressed in μg L^{-1}, did not exceed the value 0.943 μg L^{-1} (MC-RR July 2012), indicating that the water can be classified as low risk for recreational use, as far as total MCs are concerned (WHO, 2003).

The fact that the cyanobacterial content in Lake Yerevan waters exceeds the guidelines of WHO for safe practice in managing recreational waters (WHO, 2003), as well as the presence of cyanotoxins in lake biomass during 2012, should be a warning to local administration and health authorities of Armenia, to control the reservoir's water quality and to prevent the continuing transformation of the reservoir from meso-to deep eutrophic stage. Additionally, the concentrations of MCs in the waters of Lake Yerevan (November 2016) and in pure cyanobacterial cultures were low but they demonstrated that Lake Yerevan waters present a potential risk of cyanotoxin production, which could increase under favorable conditions for cyanobacteria proliferation.

Lake Yerevan is a part of a long water cascade that finally flows

Table 2
Study of cyanotoxins' occurrence in various water bodies of South-East Europe.

Country	Sampling Period	Water body	Cyanotoxin detected	Range of Concentration	Method of analysis	Reference
Bulgaria	August 2004	Water bodies (Shabla, Ezeretz, Durankulak, Vaya, Yasna Polyana, Mandra, Iskur, Studena, Pchelina, Choklyovo Blato, Bistritsa, Dolni Borgov, Botunets, Kutina, Druzhba)	MC-RR, MC-LR, MC-YR	Intracellular content Total MCs: 8–1070 ng mg^{-1} dw MC-LR: trace – 260 ng mg^{-1} dw	HPLC-DAD	Pavlova et al., 2006
Bulgaria	July and September 2006	Borovitsa	ANA, Total MCs (MC-LR, MC-LA, MC-RR, MC-YR)	Extracellular content: Total MCs and Nodularins: 0.09 –0.18 μg L^{-1} ANA: (detected but not quantified)	HPLC-UV ELISA	Teneva et al., 2010
Bulgaria	2011	Golyamo Skalensko Lake Malko Skalensko Lake	Total MCs	Extracellular content: Total MCs and Nodularins: non-detected - 400 μg L^{-1}	HPLC-UV ELISA	Teneva et al., 2014
Bulgaria	2000–2015	120 water bodies including Reservoirs Bistritsa, Borovitsa, Enitsa, Kayabash, Krushovitsa, Mandra, Pchelina, Studena, Studen kladenets, Trakiets, Vucha, Valchovets Lakes Vaya, Durankulashko ezero, Ezero Momin brod	MC-LR, MC-LA, MC-RR, MC-YR, nodularins, ANA and saxitoxins	Extracellular content: Total MCs: 0.018–26.5 μg L^{-1} Intracellular biomass content: Total MCs: trace – 1070 ng mg^{-1}	HPLC-DAD LC-MS ELISA	Stoyneva-Gärtner et al., 2017
Greece	2007–2010	Lake Marathonas	MC-RR, MC-YR, MC-LR, MC-LA	Extracellular MCs: MC-RR 0.002–0.174 μg L^{-1} MC-YR up to 0.717 μg L^{-1} MC-LR 0.002–0.451 μg L^{-1} MC-LA up to 0.008 μg L^{-1} Intracellular MCs (one biomass sample): MC-RR 1956 ng mg^{-1} MC-YR 555 ng mg^{-1} MC-LR 382 ng mg^{-1}	LC-MS/MS	Kaloudis et al., 2013
Greece	2014–2015	Lake Kastoria Lake Marathonas	[DAsp^3]MC-RR, MC-RR, MC-YR, [DAsp^3]MC-LR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY	Extracellular MCs [DAsp^3]MC-RR 1.7 μg L^{-1} MC-RR 63 μg L^{-1} MC-YR 0.050–3.6 μg L^{-1} [DAsp^3]MC-LR 0.004–0.48 μg L^{-1} MC-LR 0.063–18 μg L^{-1} MC-HilR up to 0.42 μg L^{-1} MC-WR up to 0.51 μg L^{-1} MC-LA up to 0.54 μg L^{-1} MC-LY up to 0.16 μg L^{-1}	LC-MS/MS	Zervou et al., 2017a
Greece	2007–2016	from 14 different lakes and surface water reservoirs in Greece: Pamvotis, Kastoria, Mikri Prespa, Petron, Chimaditis, Zazari, Vegoritis, Doirani, Kerkini, Volvi, Vistonis, Ismarida, Marathonas, Trichonis	CYN, ANA-a, STX, neoSTX, [D-Asp^3]MC-RR, MC-RR, MC-YR, MC-HtyR, [D-Asp^3]MC-LR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW and MC-LF	Intracellular MCs CYN: up to 1.72 ng mg^{-1} ANA up to 61.7 ng mg^{-1} [DAsp^3]MC-RR up to 175 ng mg^{-1} MC-RR up to 754 ng mg^{-1} MC-YR up to 128 ng mg^{-1} MC-HtyR up to 2.94 ng mg^{-1} [DAsp^3]MC-LR up to 26.8 ng mg^{-1} MC-LR up to 458 ng mg^{-1} MC-HilR up to 18.7 ng mg^{-1} MC-WR up to 12.7 ng mg^{-1} MC-LA up to 1.32 ng mg^{-1} MC-LY up to 8.73 ng mg^{-1} MC LW up to 2.94 ng mg^{-1} MC-LF up to 2.50 ng mg^{-1}	LC-MS/MS	Zervou et al., 2017b
Greece	1996–2004	36 freshwater bodies	Total MCs	For Lakes Kastoria and Pamvotis: Total MCs up to 13230 μg L^{-1}	ELISA HPLC-DAD PP1 Inhibition Assay	Gkelis et al., 2015
Romania	2001	Gheorgheni recreational water body	MC-LR, MC-RR, MC-YR, MC-WR, [D-Asp^3]MC-LR	Intracellular content: Total MCs: 199–2875 ng mg^{-1}	ELISA MALDI-TOF	Boaru et al., 2006
Russia	August 2009	Lake Kotokel	MC-RR, MC-LR, MC-YR	Intracellular content: Total MCs: 53 ng mg^{-1}	HPLC-UV	Belykh et al., 2011
Lithuania/ Russia	2014	Curonian Lagoon	MC-LR, MC-RR, MC-LF, MC-LY, MC-LW, MC-YR, dMC-RR	Intracellular content: Total MCs: 0.52–153.60 μg L^{-1}	LC-MS/MS	Šulčius et al., 2015
Serbia	2007	Seven lakes in Vojvodina	Total MCs	Intracellular content: Total MCs: 1.1–238 μg L^{-1}	PP1 Inhibition Assay	Svirčev et al., 2013
Serbia	2000–2017		Total MCs			Svirčev et al., 2017

(*continued on next page*)

Table 2 (*continued*)

Country	Sampling Period	Water body	Cyanotoxin detected	Range of Concentration	Method of analysis	Reference
		Review of 65 aquatic ecosystems and reservoirs listed in the Serbian Cyanobacterial Database		Total content in MC-LR eq.: Canals up to 347 $\mu g\ L^{-1}$ Rivers: 22 $\mu g\ L^{-1}$ - 4.84 $mg\ L^{-1}$ Fishponds up to 181 $\mu g\ L^{-1}$ Irrigation reservoirs up to 280 $\mu g\ L^{-1}$ Lakes: maximum values 389 –604 $\mu g\ L^{-1}$ Drinking water reservoirs up to 650 $\mu g\ L^{-1}$	PP1 Inhibition Assay HPLC-UV ELISA	
Turkey	2013	Lake Iznik	CYN	Intracellular CYN: non detected – 3471 $\mu g\ L^{-1}$	LC-MS/MS	Akcaalan et al., 2014
Turkey	1997	Lakes Sapanca, Iznik and Taskisi (Calticak)	Total MCs, MC-RR, MC-LR, MC-LW	Total MCs: non-detected – 4.8 $\mu g\ L^{-1}$ MC-LR eq.	HPLC-PDA ELISA PP1 Inhibition Assay	Albay et al., 2003
Turkey	2000–2003	Kucukcekmece Lagoon	Total MCs, MC-YR, MC-LR	Intracellular Total MCs: 0.1 –24.2 $\mu g\ L^{-1}$ MC-LR eq.	HPLC-PDA	Albay et al., 2005
Turkey	2015–2016	18 water bodies	Total MCs, CYN	Total MCs: 0.1–29.7 $\mu g\ L^{-1}$ CYN: 0.1–9 $\mu g\ L^{-1}$	HPLC-UV	Koker et al., 2017
Turkey	2012–2014	Lake Sapanca	MC-LR, MC-YR, MC-RR, MC-LA, MC-LY and MC-LF, Nodularin-R, CYN, ANA	CYN: up to 0.055 $\mu g\ L^{-1}$ MC-RR: up to 1.5092 $\mu g\ L^{-1}$ NOD: up to 0.0902 $\mu g\ L^{-1}$ MC-LF: up to 0.0108 $\mu g\ L^{-1}$ MC-LR: up to 1.1774 $\mu g\ L^{-1}$ MC-LY: up to 0.0080 $\mu g\ L^{-1}$ MC-YR: up to 1.4024 $\mu g\ L^{-1}$	LC-MS/MS	Greer et al., 2016

into the Caspian Sea, therefore the possibility of cyanobacteria proliferation and subsequent cyanotoxin occurrence in distant locations within this long watershed, is of great importance. In the past *Microcystis* has been demonstrated to travel long distances in Kansas River - up to 265 km, originating from a large discharge of water from distant Milford Lake (Graham et al., 2012). Similar tendency to travel over 300 km was observed in the Klamath system for *Microcystis* cells, which progressively dominated as distance increases from the reservoir (Otten et al., 2015). These studies underline the necessity for frequent and reliable monitoring of cyanobacteria and cyanotoxin occurrence in different interconnected parts of Lake Yerevan long water cascade.

5. Conclusions

This is the first report of the presence of cyanobacteria and cyanotoxins in South Caucasus region, more specifically in Armenia. Increased input of nutrients, such as nitrogen and phosphorus, along with other environmental characteristics specific for Lake Yerevan basin, i.e. water level annual decline and water temperature increase, have stimulated cyanobacterial rapid growth and bloom formation.

Although cyanotoxins in the filtered biomass from both stations in 2012 were not found at high concentrations, some of them, such as MC-RR, MC-LR and MC-WR, were identified in samples from both stations. MC-RR and MC-LR were also detected in samples during the fall season. Although cyanotoxin concentrations were low, the density of cyanobacterial cells was high, especially during 2013, corresponding to high probability of adverse health effects based on the guidelines set by WHO for surface waters used for recreational purposes. Since Lake Yerevan is used for irrigation, fishing, as well as for water sport activities, it may present a potential risk to human health, being in contact with the reservoir water.

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